E.Z.N.A.® Soil DNA Kit

D5625-00	5 preps
D5625-01	50 preps
D5625-02	200 preps

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OMEGA bio-tek

Innovations in nucleic acid isolation

Introduction

The E.Z.N.A.® Soil DNA Kit allows rapid and reliable isolation of high-quality genomic DNA from various soil samples. Up to 1 gram of soil samples can be processed in less than 60 minutes. The system combines the reversible nucleic acid-binding properties of HiBind® matrix with the speed and versatility of spin column technology to eliminate PCR inhibiting compounds such as humic acid from soil samples. Purified DNA is suitable for PCR, restriction digestion, and hybridization techniques. There are no organic extractions thus reducing plastic waste and hands-on time to allow multiple samples to be processed in parallel.

Overview

If using the E.Z.N.A.® Soil DNA Kit for the first time, please read this booklet to become familiar with the procedure. Soil sample is homogenized and then treated in a specially formulated buffer containing detergent. Humic acid, proteins, polysaccharides, and other contaminants are subsequently precipitated after a heat-freeze step. Contaminants are further removed by extraction steps. Binding conditions are then adjusted and the sample is applied to an HiBind® DNA Mini Column. Two rapid wash steps remove trace contaminants and pure DNA is eluted in water or low ionic strength buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

New in this Edition:

- Equilibration Buffer (used in the Troubleshooting section) is no longer included with this kit.
- Equilibration Buffer can be replaced with 3M NaOH provided by the user.

Kit Contents

Product Number	D5625-00	D5625-01	D5625-02
Purifications	5 preps	50 preps	200 preps
HiBind® DNA Mini Columns	5	50	200
2 mL Collection Tubes	10	100	400
Glass Beads	3 g	30 g	110 g
HTR Reagent	1.2 mL	12 mL	45 mL
SLX-Mlus Buffer	6 mL	60 mL	220 mL
DS Buffer	0.6 mL	6 mL	22 mL
P2 Buffer	3 mL	25 mL	60 mL
XP1 Buffer	4 mL	40 mL	160 mL
Elution Buffer	3 mL	30 mL	120 mL
SPW Wash Buffer	2 mL	20 mL	3 x 25 mL
User Manual	✓	✓	✓

Storage and Stability

Most components of the E.Z.N.A.® Soil DNA Kit should be stored at 22-25°C. Store the HTR Reagent at 2-8°C. Under these conditions, DNA has successfully been purified and used for PCR after 24 months of storage. During shipment, or storage in cool ambient conditions, precipitates may form in some buffers. It is possible to dissolve such deposits by warming the buffer to 55°C.

Preparing Reagents

Dilute SPW Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D5625-00	8 mL
D5625-01	80 mL
D5625-02	100 mL per bottle

E.Z.N.A.® Soil DNA Protocol

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000 x *g* and 4°C
- Centrifuge with rotor for 15 mL centrifuge tubes
- Vortexer
- 1.5 mL microcentrifuge tubes
- 2 mL microcentrifuge tubes
- 15 mL centrifuge tubes
- Incubator capable of 70°C
- 100% ethanol
- Isopropanol
- Optional: Incubator capable of 95°C

Before Starting:

- Prepare the SPW Wash Buffer as instructed in the "Preparing Reagents" section on Page 4
- Heat Elution Buffer to 70°C
- Set a incubator to 70°C
- Prepare ice bucket
- Set a microcentrifuge to 4°C
- Optional: For gram-positive bacteria, set a incubator or water bath to 95°C

1. Transfer 500 mg glass beads to a 15 mL centrifuge tube.

2. Add 0.2-1.0 g soil sample to the glass beads.

3. Add 1 mL SLX-Mlus Buffer. Vortex at maximum speed for 3-5 minutes to lyse samples.

   Note: For best result, a mixer mill, such as GenoGrinder 2010, Fastprep-24®, Mixer Mill MM 300®, should be used.

4. Add 100 µL DS Buffer. Vortex to mix thoroughly.

5. Incubate at 70°C for 10 min. Briefly vortex the tube once during the incubation.

Optional: For isolation of DNA from gram-positive bacteria, do a second incubation at 95°C for 2 minutes.

6. Centrifuge at 3,000 rpm for 3 minutes at room temperature.

7. Transfer 800 µL the supernatant into a new 2 mL microcentrifuge tube.

8. Add 270 µL P2 Buffer. Vortex to mix thoroughly.

9. Incubate on ice for 5 minutes.

10. Centrifuge at ≥13,000 x *g* for 5 minutes at 4°C.

11. Carefully transfer the supernatant to a new 2 mL microcentrifuge tube.

12. Add 0.7 volumes isopropanol. Mix thoroughly by inverting tube for 20-30 times.

    Note: If the soil contains very low DNA, incubate the sample at -20°C for 1 hour.

13. Centrifuge at ≥13,000 x *g* for 10 minutes at 4°C.

14. Carefully aspirate and discard the supernatant. Do not disturb the DNA pellet.

15. Invert the tube on a absorbent paper for 1 minute to drain the liquid.

    Note: It is not necessary to dry the DNA pellet.

16. Add 200 µL Elution Buffer. Vortex for 10 seconds.

17. Incubate at 70°C for 10-20 minutes to dissolve the DNA pellet.

18. Add 100 µL HTR Reagent. Vortex to mix thoroughly.

    Note: Completely resuspend HTR Reagent by shaking the bottle before use.

19. Let sit at room temperature for 2 minutes.

20. Centrifuge at ≥13,000 x *g* for 2 minutes.

21. Transfer cleared supernatant to a new 2 mL microcentrifuge tube.

    Note: If supernatant still has a dark color from the soil, repeat Steps 18-20 for a second HTR Reagent step.

22. Add an equal volume XP1 Buffer. Vortex to mix thoroughly.

23. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube provided in this kit.

24. Transfer the sample from Step 22 to the HiBind® DNA Mini Column.

25. Centrifuge at 10,000 x *g* for 1 minute at room temperature.

26. Discard the filtrate and reuse the Collection Tube.

27. Add 300 µL XP1 Buffer.

28. Centrifuge at 10,000 x *g* for 1 minute.

29. Discard the filtrate and the Collection Tube.

30. Transfer the HiBind® DNA Mini Column into a new 2 mL Collection Tube.

31. Add 700 µL SPW Wash Buffer.

    Note: SPW Wash Buffer must be diluted with ethanol before use. Please see the "Preparing Reagents" section on Page 4 for instructions.

32. Centrifuge at 10,000 x *g* for 1 minute.

33. Discard the filtrate and reuse the Collection Tube.

34. Repeat Steps 31-33 for a second SPW Wash Buffer wash step.

35. Centrifuge the empty HiBind® DNA Mini Column at ≥13,000 x *g* for 2 minutes at room temperature.

    Note: This step is critical in removing residual ethanol that may interfere with downstream applications.

36. Transfer the HiBind® DNA Mini Column into a clean 1.5 mL microcentrifuge tube.

37. Add 30-100 µL Elution Buffer heated to 70°C directly onto the center of HiBind® membrane.

38. Incubate at 70°C for 10-15 minutes.

39. Centrifuge at ≥13,000 x *g* for 1 minute.

40. Repeat Steps 37-39 for a second elution step.

41. Discard the HiBind® DNA Mini Column and store eluted DNA at -20°C.

E.Z.N.A.® Soil DNA Protocol - Purification of DNA Isolated using Other Methods

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000 x *g* and 4°C
- 1.5 mL microcentrifuge tubes
- 2 mL microcentrifuge tubes
- Incubator capable of 70°C
- 100% ethanol

Before Starting:

- Prepare the SPW Wash Buffer as instructed in the "Preparing Reagents" section on Page 4.
- Heat Elution Buffer to 70°C
- Set a incubator to 70°C
- Set a microcentrifuge to 4°C

1. Adjust the volume of the DNA sample to 200 µL with Elution Buffer.

2. Add 100 µL HTR Reagent. Vortex to mix thoroughly.

   Note: Completely resuspend HTR Reagent by shaking the bottle before use.

3. Let sit at room temperature for 2 minutes.

4. Centrifuge at ≥13,000 x *g* for 2 minutes.

5. Transfer cleared supernatant to a new 2 mL microcentrifuge tube.

   Note: If supernatant still has a dark color from the soil, repeat Steps 2-4 for a second HTR Reagent step.

6. Follow Steps 22-41 beginning on Page 7 of the Soil DNA Protocol.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, **at 1-800-832-8896.**

Problem	Cause	Solution
A260/230 ratio is low	Inefficient elimination of inhibitory compounds	Repeat with a new sample, be sure to mix the sample with HTR Reagent thoroughly.
	Salt contamination	Make sure the column is dried before the elution. Wash the column with extra SPW Wash Buffer.
Low DNA Yield or no DNA Yield	Poor homogenization of sample	Repeat the DNA isolation with a new sample, be sure to vortex the sample with SLX-Mlus and glass beads thoroughly.
	DNA washed off.	SPW Wash Buffer must be diluted with ethanol before use.
	Column matrix lost binding capacity during storage	Add 100 µL 3M NaOH to the column prior to loading the sample. Centrifuge at 10,000 x *g* for 30 seconds. Discard the filtrate.
Problems in downstream applications	BSA not added to PCR mixture	Add BSA to a final concentration of 0.1 µg/mL to the PCR mixture.
	Too much DNA inhibits PCR reactions	Dilute the DNA used in the down-stream application if possible.
	Non-specific bands in downstream PCR	Use hot-start Taq polymerase mix-ture
	Inhibitory substance in the eluted DNA.	Check the $A_{260}/_{230}$ ration. Dilute the elute to 1:50 if necessary
	Residual ethanol in the elute	Completely dry the column before elution
Little or no supernatant after initial centrifuge step	Insufficient centrifugal force	Check the centrifugal force and increase the centrifugal time if necessary
Sample can not pass through the column	Clogged column	Check the centrifugal force and increase the time of centrifugation

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
HiBind® DNA Mini Columns, 200 columns	DNACOL-02
P2 Buffer, 60 mL	PD076
SPW Wash Buffer, 40 mL	PDR045
Elution Buffer, 100 mL	PDR048

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Notes: